IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Xiaoyang Qi	: Paper No:	
Serial No.	10/801,517	: Group Art Unit:	1643
Filed:	March 16, 2004	: Examiner:	Sang, Hong
For:	SAPOSIN C-DOPS: A NOVEL ANTI-TUMOR AGENT		

Confirmation No. 4062

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This declaration under 37 CFR Sec. 1.132 is supportive of the Amendment and Response filed herewith. I, Xiayang Qi, declare and say:

1. That I am a citizen of the United States and that I am the inventor of the above-referenced patent application; that I am employed by Cincinnati Children's Medical Center as a Research Assistant Professor, and I was and still am, engaged in a research program in the field of biochemistry and molecular biology of saposins.

2. That I am familiar with the above-identified patent application Ser. No. 10/801,517, that I have reviewed the May 1, 2008 Office Action in the above captioned case; and that I am familiar with the following reference: *Vaccaro et al.* (FEBS 1993, 336(1): 159-162) in view of the teachings of *O'Brien et al.* (W09503821A1), as evidenced by *Vaccaro et al.* (FEBS, 1994, 349: 181-186).

3. That I contend that the in the rejection the Examiner states that the teachings of Vaccaro and O'Brien show forming liposomal vesicles and then adding saposin C to the formulation, resulting in a surface interaction of the protein with the vesicles. A lipid/saposin vesicle formed by the methods described in these references will not function the same *and* will not exhibit anti-tumor activity as with the vesicles of the present invention. That adding Saposin C protein to vesicles after formation would lead to merely a surface interaction, which has been found to lack the cytotoxic effect of the materials shown in the present invention wherein the polypeptide is embedded within the lipids of the vesicle.

This effect has been shown experimentally as follows:

Method 1: Sample 1 preparation (According to the methods described in the Vaccaro reference).

Buffer preparation

Buffer A: 10 mM Acetate Buffer (PH 5.8)
150 mM NaCl
1 mM EDTA

Buffer B: 150 mM NaCl
1 mM EDTA
1 mM dTT

1. Vesicle preparation:

   (A) 1mg of DOPS (dissolved in chloroform 1mg/ml) was dried under Nitrogen then dispersed in 250 µl of Buffer B (final concentration is 4 mg/ml).
   (B) The lipid suspension was submitted to sonication under nitrogen in a Branson B 15 sonifier at 30°C (3 min with a Cup Horn at a power setting of 100W, followed by 6 min with a Microtip at a power setting of 30W)
   (C) The preparation was centrifuged at 110,000 g for 30 min and the supernatant used for experiment.

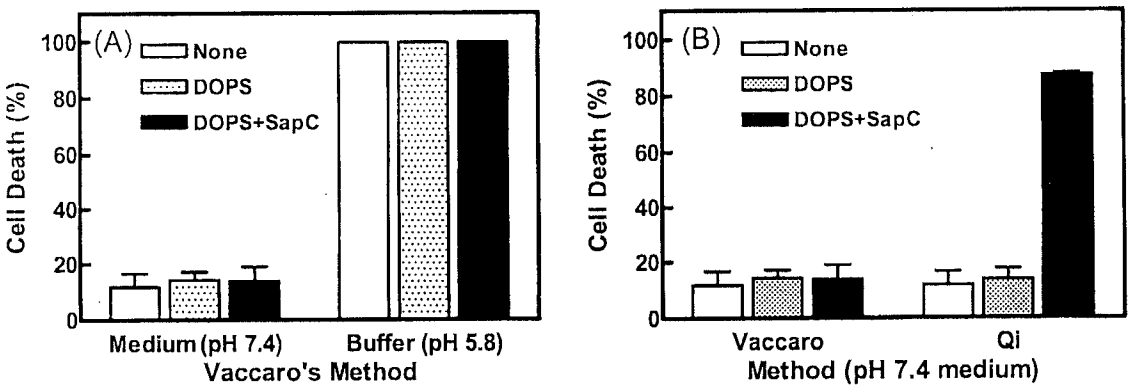
2. Add 61.2 µl of DOPS (245 µg) vesicle into 1 ml of Buffer A (pH = 5.8) or medium (pH = 7.4), then add 1.18 mg of Saposin C incubate 30 min at 30°C.

Method 2: Sample 2 (Saposin C-DOPS nanovesicle) preparation (According to methods and materials according to the present application by Xiaoyang Qi).

1. Dry 245 µg of DOPS which is in chloroform/methanol with nitrogen in glass tube.
2. Add 1.18 mg of Saposin C onto try DOPS film which is on the bottom of the glass tube.
3. Add 50 µl of C/P buffer (pH = 4.7) to the glass tube, then add 1 ml of medium (pH = 7.4) to the glass tube.
4. Sonicate the complexes 15-20 minute in ice water.

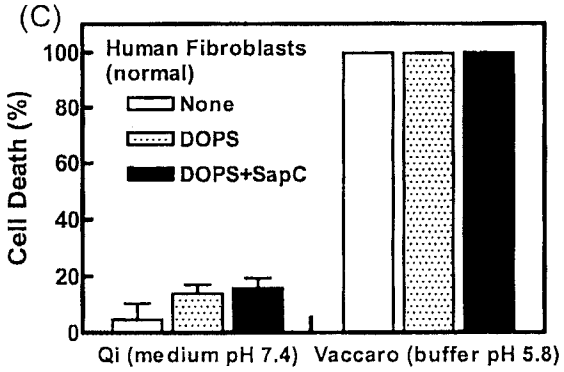
Human Cancer Cells

Experimental conditions: Human neuroblastoma cells (CHLA-20, 4 x 10^4/100 µl/well) were cultured for 24 h prior to the treatment. MTT assay was carried out after cultured the treated cells for 3 days. Spectrophotometric data from quadruplication wells (in 96 well plates) were analyzed by ANOVA. The data were presented as the arithmetic mean ± SEM.
Concentrations: SapC = 100 µM, DOPS = 300 µM.



Human Normal Cells

Experimental conditions: Human fibroblast Cells (1 x 10^4/100 µl/well) were cultured for 24 h prior to the treatment. MTT assay was carried out after cultured the treated cells for 3 days. Spectrophotometric data from quadruplication wells (in 96 well plates) were analyzed by ANOVA. The data were presented as the arithmetic mean ± SEM.
Concentrations: SapC = 100 µM, DOPS = 300 µM.



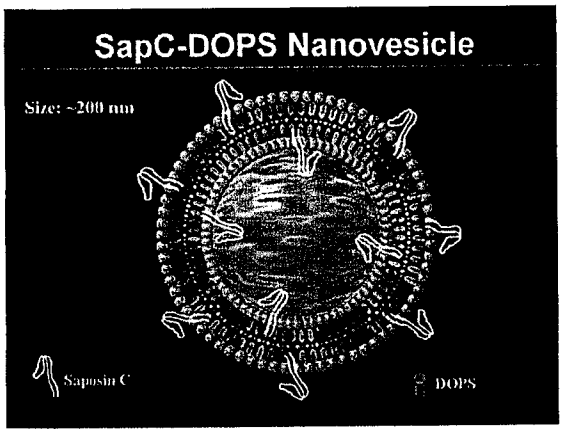
Conclusion:
Sample 1 (DOPS+SapC, **Voccaro**) prepared from Method 1 with Buffer A (pH = 5.8) kills normal and cancer cells (**Figures A and C**). Control samples [no Saposin C/DOPS (None) and DOPS alone (DOPS)] prepared from Method 1 with Buffer A (pH = 5.8) also killed normal and cancer cells (**Figures A and C**). However, if replace Buffer A with medium (pH = 7.4) in method 1, no killing effect was observed from sample 1 on cancer cells (**Figures A and B**). This indicates that acidic pH had negative effect on the cell viability.
Sample 2 (DOPS+SapC, **Qi**) prepared from Method 2 with medium (pH = 7.4) killed cancer cells, but not normal cells (**Figures B and C**). Control samples [no Saposin C/DOPS (None) and DOPS alone (DOPS)] prepared from Method 2 with medium (pH = 7.4) had no killing effect on cancer and normal cells (**Figures B and C**). This indicates sample 2's cancer-selective killing activity.

4. That within the present application, each specific example listed in the Examples builds upon the earlier one(s), incorporating all of the same information regarding materials and methods. For example, the description in Example 4 showing the *ex vivo* analysis of effects of saposin C-DOPS on SCC incorporates the tissue culture conditions of Example 3 and the bath sonication of Saposin C and dioleoylphosphatidylserine of Example 2.

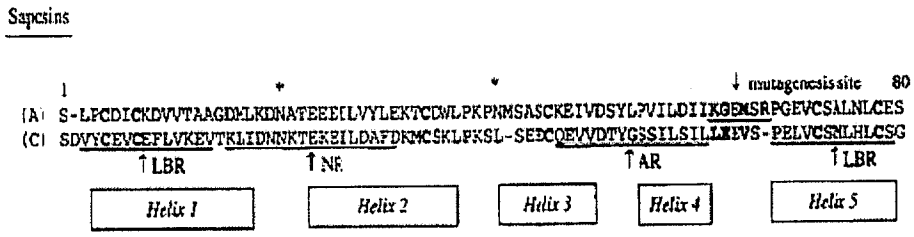
5. That the present invention is not a case of simply a composition comprising a mixture of lipid nanovesicles and polypeptide but is a composition comprising a Saposin-C-DOPS nanovesicle complex. That the composition of the present invention comprises a Saposin-C-DOPS nanovesicle complex and that it would be clear to one skilled in the art from the description of the present invention within the specification, especially as described in

Example 2, that the composition comprises a Saposin-C-DOPS nanovesicle complex and not a mixture of nanovesicles and Saposin-C suspended in a carrier.

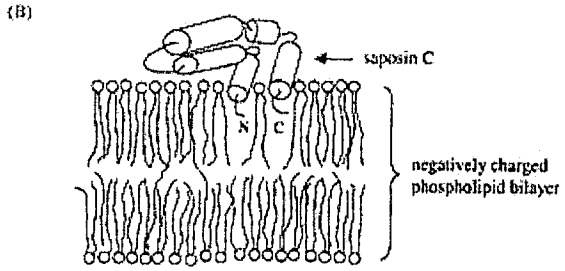
The complex can be graphically depicted as follows:



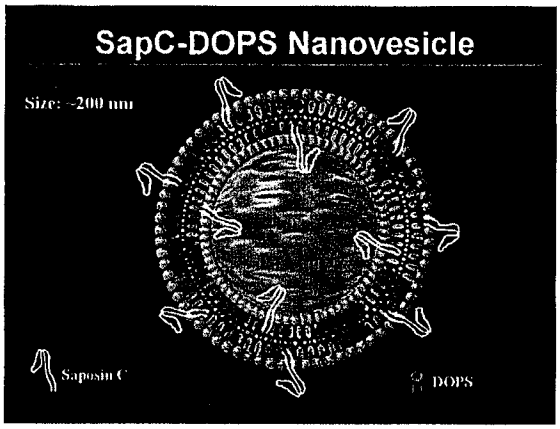
6. That the specification, taken as a whole with the art known at the time of filing, would enable one skilled in the art to understand that the present invention provides for vesicles having Saposin C embedded into the outer leaflets. As described in the specification as well as the references *J. Biol. Chem.*, Vol. 271, No. 12, pp. 6874–6880, 1996, and *J. Biol. Chem.*, Vol. 276, No. 29, pp. 27010–27017, 2001, the H-1 and H-5 helices are integral to this process, suggesting that proper membrane interaction of saposin C affects its specificity and activity. The sequence and helices are shown below:



7. That the reference J. Biol. Chem., Vol. 276, No. 29, pp. 27010–27017, 2001, describes the structure of the Saposin C molecule as having 5 helices, which was well-defined at the time the present application was filed. That the materials formed by the methods of the current application provide for the Saposin C to embed into the lipid membrane as such:



Forming a vesicle as:



8. That it is well within the capability of one of ordinary skill in the art, through routine laboratory procedures, to ascertain whether or not a given polypeptide retains plasma-membrane affinity and whether or not a given nanovesicle exhibits anti-tumor activity.

9. That it is well known in the art that the proteins of the invention may be altered in various ways including the amino acid substitutions, deletions, truncations, and insertions. Moreover, applicants have provided sufficient detail of particular patentable embodiments and a person skilled in the art can easily ascertain the sequences that fall within the scope of the present claims given that whether or not a polypeptide falls within the scope of the present claims.

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Further declarant sayeth not.

Dr. Xiayang Qi

10/30/08

Date